EXERCISE 3 - Postlab

A. pH and Buffers

1. Following the procedure described in the Prelab, plot a titration curve for each of the following solutions:

   20 mM sucrose in dH_2O

   20 mM sucrose in an acetate buffer with a pH of 4.8

   20 mM sucrose in a bicarbonate buffer with a pH of 9.8

   Plot each curve on a separate sheet of paper, and use the entire page for each graph. Use the "Graphing Checklist" in PreLab 2.8 to make sure you have included all necessary information on your graph.

2. How does the titration curve of the unbuffered sucrose solution compare with the titration curves of the 2 buffered solutions?

3. What is the **buffering range** of the acetate buffer? **Explain your answer.**

4. What are the **buffering ranges** of the bicarbonate buffer? **Explain your answer.**

5. Which solution, if any, seems to have the greater **buffering capacity**? **Explain your answer.**

6. Would either the acetate buffer or the bicarbonate buffer be a good choice for studying enzymes or other biomolecules found in human blood? **Explain your answer.**

7. Explain how the pH scale is used to describe the H^+ concentration of a solution.

8. Describe what a buffer is and explain why buffers are important to living organisms.

9. You wish to study a protein found in cow's milk called *α-lactalbumin*. You measure the pH of cow's milk and find that it is 6.0. You then check a biochemistry reference manual to identify a suitable buffer for use during purification and study of this enzyme. Your research indicates that phosphate buffer has a buffering range of pH 5.7 to pH 8.0. Further, to prepare a phosphate buffer with a pH of 6.0, you need a solution that contains 87.7 mM monobasic sodium phosphate (FW = 137.99) and 12.3 mM dibasic sodium phosphate (FW = 141.96). You also want your buffer to contain 0.02% thimerasol, a biocide that will prevent the growth of bacteria, mold, and other organisms in the buffer. Write a short paragraph describing ***exactly*** how you would prepare 900 mL of this buffer. Describe your actions–exactly what you would ***do*** –step-by-step– when preparing this buffer. Also show all calculations. Be sure all amounts include units of measurement.

B. Introduction to the Brightfield Microscope

1. Answer each of the following questions regarding the brightfield microscope:

   a. What is the diaphragm used for?
   b. What is the condenser used for?
   c. What are the magnifications of your objective lenses, and what is the total magnification of the microscope when using each lens?
   d. What does parfocal mean?
   e. Why is it important to focus your specimen on low power first, before switching to high power?

2. What is meant by **field of view**? Explain what happens to the field of view as you increase the magnification of the objective lens.

3. What is meant by **depth of field**? Explain what happens to the depth of field as you increase the magnification of the objective lens.

4. Explain why *Euglena* seem to move so much faster when viewed at high magnification than when viewed at low power.

5. Living *Euglena* are quite visible by brightfield microscopy without staining or other special preparation. Give a possible reason for their visibility.

6. Based on your observations, list at least one advantage that a permanent dry mount has over a wet mount for examining *Euglena* by brightfield microscopy.

7. Based on your observations, list at least one advantage that a wet mount has over a permanent dry mount for examining *Euglena* by brightfield microscopy.